Relationship of Mitotic Arrest and Apoptosis to Antitumor Effect of Paclitaxel

Christopher G. Milross, Kathryn A. Mason, Nancy R. Hunter, Woong-Ki Chung, Lester J. Peters, Luka Milas*

Background: Microtubules are cellular organelles with functions that include control of cell division by mitosis, cell morphology, and transport of material within the cell. The anticancer drug paclitaxel (Taxol) promotes accelerated assembly of excessively stable microtubules. Consequently, treated cells tend to become arrested in mitosis. The drug also induces apoptotic cell death in vitro and in vivo. Prior to this study, the relative contributions of mitotic arrest and apoptosis to the in vivo antitumor effect and the relationship between the two factors had not been established; moreover, it is not known whether paclitaxel-induced mitotic arrest inevitably results in cell death. Purpose: Our aim was to quantify the mitotic arrest and apoptosis induced by paclitaxel in 16 murine tumors in vivo and to correlate these two factors with the drug's antitumor effect. Methods: Inbred C3Hf/Kam mice were implanted with one of the following 16 syngeneic tumors: seven adenocarcinomas (MCa-4, MCa-29, MCa-35, MCa-K, OCa-I, ACa-SG, and HCa-I), two squamous cell carcinomas (SCC-IV and SCC-VII), six sarcomas (FSA, FSa-II, Sa-IIa, Sa-NH, NFSa, and Sa-4020), and one lymphoma (Ly-TH). The tumor growth delay induced by paclitaxel (40 mg/kg body weight given intravenously) was measured in 163 control and 163 treated mice, and its significance was assessed by Student's t-test. In a separate group of 439 mice, the percentage of cells in mitosis or apoptosis was scored micromorphometrically at various times after paclitaxel administration. The significance of correlations between paclitaxel-induced tumor growth delay and paclitaxel-induced levels of mitosis or apoptosis was determined by simple correlation and Spearman's rank correlation. P values reported represent two-sided tests of statistical significance. Results: Statistically significant tumor growth delays were found in response to paclitaxel treatment of mice for three of four murine mammary carcinomas (all P<.01), an ovarian carcinoma (P = .00003), a salivary gland adenocarcinoma (P = .0002), a lymphoma (P = .0002), and two of six sarcomas (both P<=.034), but not for either of two squamous cell carcinomas or for the hepatocellular carcinoma. Paclitaxel-induced mitotic arrest was apparent in all tumor types, but to various degrees, and was not significantly correlated with growth delay (R^2 = .16; P = .124). In contrast, apoptotic cell death in response to paclitaxel was not ubiquitous, but it was strongly correlated with growth delay (R^2 = .59; P = .001). The pretreatment level of apoptosis was correlated with both paclitaxel-induced apoptosis (R^2 = .71; P = .00004) and tumor growth delay (R^2 = .55; P = .001). Conclusion: The antitumor effect of paclitaxel was correlated with paclitaxel-induced apoptosis and base-line apoptosis, but not with mitotic arrest. Implications: Apoptosis is an important mechanism of cell death in response to paclitaxel treatment of in vivo murine tumors. An underlying tumor type-specific propensity for apoptosis is implied by the correlation between pretreatment and paclitaxel-induced apoptosis. Both the extent of pretreatment apoptosis and the paclitaxel-induced percentage of apoptosis may be useful predictors of response to the drug. [J Natl Cancer Inst 1996;88:1308-14]

*Affiliation of authors: Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston.

Correspondence to: Luka Milas, M.D., Ph.D., Department of Experimental Radiation Oncology, Box 066, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030.

See “Notes” section following “References.”

Microtubules are cellular organelles with functions that include control of mitosis, morphology, and intracellular transport (1). The anticancer drug paclitaxel promotes accelerated assembly of excessively stable microtubules (2). Consequently, treated cells tend to become arrested in mitosis (3-5). Paclitaxel also induces apoptotic cell death both in vitro (6,7) and in vivo (8,9). The relative contributions of mitotic arrest and apoptosis to the in vivo antitumor effect and the relationship between the two have not been established, and it is not known whether paclitaxel-induced mitotic arrest inevitably results in cell death.

Elucidation of the relationship between paclitaxel-induced mitotic arrest and apoptosis and the relative contribution of both to the antitumor effect may facilitate the use of this drug singly or in combination with other cytotoxic agents (in particular, radiotherapy). For example, induction of mitotic arrest without associated cell destruction would theoretically make paclitaxel ineffective on its own but might greatly enhance radiation treatment because mitotic cells are more radiosensitive than cells in other phases of the cell cycle (10-12). In vitro studies (4,13-17) have shown that paclitaxel enhances the radioresponse of most cell lines. In cases where paclitaxel was tested, cell lines showed considerable G2/M block at the time of their exposure to radiation (4,13,15,17).

Our earlier studies (8,9) on the antitumor effect of paclitaxel in murine tumor systems in vivo showed that tumors responding to the drug by growth delay histologically displayed both mitotic arrest and apoptosis. In one of these tumors, the MCA-4 mammary carcinoma, paclitaxel enhanced the radiation response (18,19). An increase in the radiation enhancement factor for tumor growth delay from 1.2 to 1.9 was observed when the interval between paclitaxel administration and subsequent radiation treatment was increased from 9 to 48 hours (19). Additional investigations established that the major underlying mechanism of this radioenhancement was not the increased cellular radiosensitivity of mitotically arrested cells but tumor reoxygenation attributable to loss and removal of cells via paclitaxel-induced apoptosis (19).

The aim of our study was to quantify the mitotic arrest and apoptosis induced by paclitaxel in 16 murine tumors in vivo and...
and to correlate these two factors with the drug's antitumor effect.

Materials and Methods

Mice

Inbred male C3H/Ka mice from our own specific-pathogen-free mouse colony were used. They were 3-4 months old and weighed from 27 to 34 g (mean weight, 32 g). The mice were housed five or six per cage and fed sterilized pelleted food (Prolab Animal Diet; Agway Inc., Syracuse, NY) and sterilized water ad libitum. The experimental protocol was approved by, and in accordance with, institutional guidelines established by the Institutional Animal Care and Use Committee. Animals were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the U.S. Department of Agriculture and U.S. Department of Health and Human Services.

Tumors

Solitary tumors were generated in the muscle of the right leg of the mouse by inoculation of 5 x 10³ viable tumors cells in suspension. Tumor cell suspensions were prepared by mechanical disruption and enzymatic digestion of non-necrotic tumor tissue. The method has been fully detailed previously (20).

We studied the following tumors: seven adenocarcinomas (four mammary carcinomas, designated MCA-4, MCA-29, MCA-35, and MCA-K, used in their fourth isotransplant generations, or fifth in the case of MCAK; an ovarian carcinoma, designated OCA-I, used in its seventh isotransplant generation; a salivary gland adenocarcinoma, designated ACA-SG, used in its sixth isotransplant generation; and a hepatocellular carcinoma, designated HCA-I, also used in its sixth isotransplant generation), two squamous cell carcinomas (designated SCC-IV and SCC-VII, used in their sixth isotransplant generation), six soft-tissue sarcomas (designated FSA, FSA-II, Sa-IIa, Sa-NH, NFA, and Sa-4020, used in their eighth, sixth, sixth, thirteenth, and seventh isotransplant generations, respectively), and one lymphoma (designated Ly-TH, used in its seventh isotransplant generation). All tumors were syngeneic to this strain of mouse. The MCA-4, MCA-29, MCA-35, ACA-SG, HCA-I, SCC-VII, FSA-II, Sa-IIa, Sa-NH, and Ly-TH tumors originally developed spontaneously, and the MCA-K, OCA-I, NFA, and Sa-4020 tumors developed developed outside the treated volume in irradiated mice. The SCC-IV and FSA tumors were induced by 3-methylcholanthrene. Homogenates of these tumors have been maintained in liquid nitrogen. They have been studied extensively over a period of many years, and their responses to radiation and a variety of other chemotherapeutic agents have been previously reported (92/23).

Paclitaxel

Paclitaxel (Taxol; lot NBT 35981, CAS No. 33069-62-4) was supplied by Dr. John Whisnant (Baker Norton Pharmaceuticals, Miami, FL). It was dissolved in equal volumes of absolute ethanol (Aaper, Shelbyville, KY) and Cremophor EL (lot No. 53H0970; Sigma Chemical Co., St. Louis, MO), sonicated for 30 minutes, and stored at 4 °C for up to 1 week. The paclitaxel stock solution (20 mg/mL) was further diluted 1:5 with sterile physiologic saline within 10 minutes of injection. Paclitaxel was administered intravenously at a dose of 40 mg/kg body weight.

Tumor Growth Delay

The antitumor effect of paclitaxel was determined by its ability to delay tumor growth. Palpable tumors were measured daily in three orthogonal diameters with Vernier calipers. Mice whose tumors grew to 8 mm in diameter (arithmetic mean) were then allocated randomly to either the tumor growth delay arm or the histologic analysis arm of the study.

For each histologic tumor type, the effect of paclitaxel (40 mg/kg given intravenously on the day the tumors reached 8 mm in diameter) was assessed by the measurement of tumor growth in untreated control and paclitaxel-treated groups at daily intervals until tumors grew to at least 12 mm in diameter. The general experimental design for tumor growth delay was to allocate six mice to each of the control and treatment groups for each histologic tumor type. Growth delay experiments, however, were repeated on several occasions with MCA-4, OCA-I, and SCC-VII tumors, and these data were combined; in two tumors, SCC-IV and Sa-4020, only five mice were allocated to control and treatment groups. A total of 326 mice were used, 163 each in the control and the treatment arms; the exact breakdown by tumor type is given in Table 1. The effect of treatment on tumor growth was expressed as absolute growth delay, defined as the time in days for the tumors in the treated groups to grow from 8 to 12 mm in diameter minus the time in days for untreated control tumors to grow from 8 to 12 mm in diameter. A detailed description and critique of the tumor growth delay assay have been published (24).

Table 1. Antitumor effect of paclitaxel against different types of in vivo murine tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Time in days for tumor to grow from 8 to 12 mm in diameter*</th>
<th>Absolute growth delay ‡</th>
<th>P §</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control†</td>
<td>Treated†</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCA-4</td>
<td>7.1 ± 0.6 (28)</td>
<td>11.9 ± 0.6 (34)</td>
<td>4.8</td>
</tr>
<tr>
<td>MCA-29</td>
<td>7.7 ± 1.0 (6)</td>
<td>12.8 ± 1.1 (6)</td>
<td>5.1</td>
</tr>
<tr>
<td>MCA-35</td>
<td>22.5 ± 0.7 (6)</td>
<td>21.3 ± 2.3 (6)</td>
<td>-1.2</td>
</tr>
<tr>
<td>MCA-K</td>
<td>6.2 ± 0.6 (6)</td>
<td>10.7 ± 0.4 (6)</td>
<td>4.5</td>
</tr>
<tr>
<td>OCA-I</td>
<td>9.9 ± 0.6 (32)</td>
<td>22.6 ± 0.8 (26)</td>
<td>12.7</td>
</tr>
<tr>
<td>ACA-SG</td>
<td>5.4 ± 0.3 (6)</td>
<td>10.6 ± 0.7 (6)</td>
<td>5.2</td>
</tr>
<tr>
<td>HCA-I</td>
<td>6.7 ± 0.6 (6)</td>
<td>6.4 ± 0.4 (6)</td>
<td>-0.3</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC-IV</td>
<td>4.5 ± 0.3 (5)</td>
<td>5.3 ± 0.6 (5)</td>
<td>0.8</td>
</tr>
<tr>
<td>SCC-VII</td>
<td>3.9 ± 0.2 (26)</td>
<td>3.8 ± 0.1 (25)</td>
<td>-0.1</td>
</tr>
<tr>
<td>Sarcoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSA</td>
<td>3.9 ± 0.3 (6)</td>
<td>4.0 ± 0.3 (6)</td>
<td>0.1</td>
</tr>
<tr>
<td>FSA-II</td>
<td>2.3 ± 0.2 (6)</td>
<td>2.9 ± 0.2 (6)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sa-IIa</td>
<td>5.6 ± 0.8 (6)</td>
<td>8.2 ± 0.7 (6)</td>
<td>2.6</td>
</tr>
<tr>
<td>Sa-NH</td>
<td>5.7 ± 0.6 (6)</td>
<td>7.5 ± 0.4 (6)</td>
<td>1.8</td>
</tr>
<tr>
<td>NFA</td>
<td>4.6 ± 0.2 (6)</td>
<td>5.9 ± 0.8 (6)</td>
<td>1.3</td>
</tr>
<tr>
<td>Sa-4020</td>
<td>4.3 ± 0.1 (5)</td>
<td>5.0 ± 0.4 (5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ly-TH</td>
<td>4.8 ± 0.3 (7)</td>
<td>18.8 ± 1.9 (8)</td>
<td>14.0</td>
</tr>
</tbody>
</table>

*The time in days to grow from 8 to 12 mm (arithmetic mean diameter) is presented as group mean ± standard error.
†Mice bearing 8-mm tumors in the right leg were treated with paclitaxel (40 mg/kg intravenously) or allocated to control groups. Tumor growth was determined by daily measurement of three orthogonal diameters with Vernier calipers. Shown in parentheses are the number of mice used in each group.
‡Defined as the time in days for tumors treated with paclitaxel (40 mg/kg intravenously) to grow from 8 to 12 mm (arithmetic mean diameter) minus the time in days for untreated control tumors to grow from 8 to 12 mm (arithmetic mean diameter).
§The time in days to grow from 8 to 12 mm (arithmetic mean diameter) was compared for treatment and control groups by use of Student's t test. Tests were two-tailed and assumed unequal variance. P values are two-sided and were taken to be significant when ≤0.05.
Histologic Determination of Mitotic and Apoptotic Indices

At 1-, 3-, 6-, 9-, 12-, 24-, 36-, and 48-hour intervals after paclitaxel administration, three mice were killed by cervical dislocation. Their tumors were removed immediately and placed in neutral buffered formalin. For each histologic type, tumors were also removed from four untreated mice. Minimal deviation from this plan resulted in a total of 439 mice being used for this part of the experiment. The total number of mice used in the 48-hour time course is shown for each tumor type in Table 2. The tissue was embedded in paraffin blocks, and 4-μm sections were cut from these blocks and stained with hematoxylin–eosin.

The morphologic features used to identify mitosis and apoptosis in murine tumors have been described and illustrated previously (8). Five fields of non-necrotic areas were selected randomly across each tumor section; in each field, apoptotic bodies and cells in mitosis were expressed as a percentage based on the scoring of 1500 nuclei (2000 nuclei for untreated controls) at each interval after treatment. Micromorphometric scoring of histologic tumor sections was conducted without knowledge of both the tumor identity and the results of the tumor growth delay assay.

Statistical Methods

For each histologic tumor type, a plot was constructed, showing the percentage of mitotic and apoptotic bodies as a function of time after paclitaxel treatment. From these plots, we determined the base-line levels of mitosis and apoptosis in untreated tumors and the temporal change in these levels after treatment. The latter were expressed as peak level, defined as the maximum level occurring within the first 48 hours after treatment; net level, defined as the peak level minus the base-line level; and an integral measure, determined by the area under the curves.

The statistical significance of the differences in the time for untreated and treated tumors to grow from 8 to 12 mm in diameter was tested by use of Student’s t test (25). T-tests were two-tailed and assumed unequal variance; the P values are two-sided and were considered to be significant if they were less than or equal to .05. Scattered plots were used to determine the correlation between paclitaxel-induced peak levels of mitosis and apoptosis and the antitumor effect of paclitaxel (25). Data were fitted by simple linear regression. In addition, in each case, a Spearman’s rank order correlation was performed (25).

Results

The antitumor effect of paclitaxel varied considerably among tumor types (Table 1). Eight (50%) of the 16 tumors showed significant growth delay. Adenocarcinomas were the most likely to respond: five (71%) of seven showed significant growth delay. In comparison, only two (33%) of six sarcomas and zero of two squamous cell carcinomas showed significant growth delay. The growth of lymphoma was also significantly delayed.

All tumors were examined histologically for mitotic arrest and apoptosis at sequential times (1-48 hours) after paclitaxel treatment. The three methods used to quantify paclitaxel-induced mitotic arrest and apoptosis (i.e., peak level, net level, and the integral measure) were strongly correlated (all R² ≥ .69). Thus, subsequent analyses were performed with the use of the peak percentages only.

Paclitaxel induced mitotic arrest in all tumors, but the extent (mean ± standard error) of arrest varied greatly, from 6.5% ± 0.7% (Sa-NH sarcoma) to 26.2% ± 1.5% (Ly-TH lymphoma). In general, higher values of mitotic arrest were observed in the adenocarcinomas and squamous cell carcinomas than in the sarcomas. Induction of apoptosis by paclitaxel showed even greater variability, from only the slightest increase (Hca-I, SCC-VII, FSA, and NFSA) to 35% ± 3.9% apoptotic cells in Ly-TH lymphoma. After the lymphoma, the greatest induction of apoptosis was seen in the adenocarcinomas; the lowest was seen in the sarcomas.

Paclitaxel induced mitotic arrest in all tumors, but it induced apoptosis only in some (Table 2). There were three general types of response. The first type exhibited high levels of both mitotic arrest and apoptosis. The adenocarcinomas (with the exception of Hca-I) and the Ly-TH lymphoma fit this category. The second type exhibited moderate or high mitotic arrest but no apoptotic response, as exemplified by the SCC-VII squamous cell carcino-

Table 2. Effect of paclitaxel on mitosis and apoptosis in different types of in vivo murine tumors

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>Mitosis</th>
<th>Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Base line†</td>
<td>Peak‡</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>MCa-4 (29)</td>
<td>1.1 ± 0.3</td>
<td>24.3 ± 0.4 (9)</td>
</tr>
<tr>
<td></td>
<td>MCa-29 (28)</td>
<td>1.0 ± 0.2</td>
<td>15.6 ± 1.2 (9)</td>
</tr>
<tr>
<td></td>
<td>MCa-35 (28)</td>
<td>1.2 ± 0.3</td>
<td>17.3 ± 1.8 (6)</td>
</tr>
<tr>
<td></td>
<td>MCa-K (28)</td>
<td>1.4 ± 0.1</td>
<td>22.2 ± 0.7 (9)</td>
</tr>
<tr>
<td></td>
<td>OCa-I (25)</td>
<td>0.6 ± 0.1</td>
<td>14.1 ± 1.7 (9)</td>
</tr>
<tr>
<td></td>
<td>ACA-SG (28)</td>
<td>0.9 ± 0.1</td>
<td>25.1 ± 2.2 (12)</td>
</tr>
<tr>
<td></td>
<td>HCa-I (32)</td>
<td>0.9 ± 0.2</td>
<td>10.2 ± 0.9 (6)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>SCC-IV (28)</td>
<td>1.0 ± 0.3</td>
<td>20.6 ± 3.2 (9)</td>
</tr>
<tr>
<td></td>
<td>SCC-VII (28)</td>
<td>0.5 ± 0.1</td>
<td>24.7 ± 1.9 (6)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>FSA (28)</td>
<td>2.2 ± 0.2</td>
<td>10.3 ± 0.7 (6)</td>
</tr>
<tr>
<td></td>
<td>FSA-II (28)</td>
<td>1.1 ± 0.3</td>
<td>10.0 ± 1.5 (3)</td>
</tr>
<tr>
<td></td>
<td>Sa-IIa (29)</td>
<td>1.4 ± 0.2</td>
<td>12.6 ± 1.0 (9)</td>
</tr>
<tr>
<td></td>
<td>Sa-NH (28)</td>
<td>0.3 ± 0.1</td>
<td>6.5 ± 0.7 (3)</td>
</tr>
<tr>
<td></td>
<td>NFSA (29)</td>
<td>0.7 ± 0.2</td>
<td>8.3 ± 1.1 (13)</td>
</tr>
<tr>
<td></td>
<td>Sa-4020 (28)</td>
<td>0.6 ± 0.2</td>
<td>11.3 ± 1.3 (6)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Ly-TH (15)</td>
<td>2.7 ± 0.4</td>
<td>26.2 ± 1.5 (9)</td>
</tr>
</tbody>
</table>

*Mice bearing 8-mm tumors in the right leg were treated with paclitaxel (40 mg/kg intravenously). The percent of cells in mitosis or apoptosis was scored from histologic sections prepared from tumors excised 1 to 48 hours after treatment. Shown in parentheses are the number of mice used in each group.

†The base-line level of mitosis and apoptosis was that determined in untreated control tumors. Shown is the group mean ± standard error.

‡The peak level of mitosis and apoptosis was determined from the time course of treated tumors and is defined as the highest percentage observed within 48 hours. Shown is the group mean ± standard error; the time (hours) at which the peak was detected is indicated in parentheses.
ma. The third type, mainly the sarcomas, had low levels of mitotic arrest and low levels of induced apoptosis.

In the paclitaxel-sensitive mammary adenocarcinomas, in areas where mitotic arrest was prominent, apoptotic bodies appeared adjacent to the cells arrested in mitosis. In addition, some cells underwent lysis, characterized by disruption of the plasma membrane and spillage of condensed chromatin into the interstitial space, as previously observed (8). In the more resistant squamous cell carcinomas, multinucleate cells appeared after the peak of mitotic arrest. In all cases in which both mitotic arrest and apoptosis occurred, the peak of mitotic arrest preceded apoptosis by several hours. While the peak of mitotic arrest occurred 3-12 hours after paclitaxel administration, the peak of apoptosis occurred 6-36 hours later (Table 2).

To determine whether there was a relationship between paclitaxel-induced mitosis and/or apoptosis and the antitumor effect of paclitaxel, we plotted the absolute growth delay against the peak of mitotic arrest or apoptosis (Fig. 1) and calculated the correlation coefficients. The correlation plot in Fig. 1, A, shows only a slight trend toward higher mitotic arrest with greater tumor growth delay ($R^2 = .16; P = .124$). In contrast, the peak levels of apoptosis showed a highly statistically significant positive correlation with absolute tumor growth delay ($R^2 = .59; P = .001$) (Fig. 1, B). Rank order correlation (to minimize the influence of particular points) confirmed the lack of correlation between mitotic arrest and growth delay ($R^2 = .16; P = .119$) and statistically significant correlation between apoptosis and growth delay ($R^2 = .62; P = .0003$). Thus, apoptosis, but not mitotic arrest, was an underlying cellular indicator of therapeutic efficacy of paclitaxel.

In addition, we tested whether the background level of apoptosis was correlated with paclitaxel-induced apoptosis or antitumor efficacy. Fig. 2 shows that the base-line level of apoptosis was correlated with both the peak apoptosis ($R^2 = .71; P = .00004$) and the antitumor efficacy ($R^2 = .55; P = .001$). Rank order correlation (to increase the reliability of this result based on small base-line measures) confirmed that base-line levels of apoptosis were correlated with both peak levels of apoptosis ($R^2 = .44; P = .005$) and growth delay ($R^2 = .37; P = .013$). Therefore, the spontaneous level of pretreatment apoptosis was also a statistically significant cellular determinant of paclitaxel's antitumor efficacy.

**Discussion**

Three important findings emerged from our observations. First, there is a broad intertumor variability of the antitumor effect of paclitaxel. Second, paclitaxel can induce mitotic arrest in all tumor types, although this biomarker failed to show a significant correlation with the antitumor effect. Third, paclitaxel-induced apoptosis is correlated with the antitumor effect, base-line apoptosis is correlated with paclitaxel-induced peak apoptosis, and base-line apoptosis is correlated with the antitumor effect of paclitaxel.

Paclitaxel significantly delayed tumor growth in eight of the 16 tumors. The adenocarcinomas of mammary origin were the most sensitive; significant growth delay was found in three (75%) of the four tumors. In contrast, significant growth delay occurred in only two (33%) of six sarcomas. Growth delay was longer for the responding mammary adenocarcinomas (4.5-5.1 days) than for the responding sarcomas (1.8 and 2.6 days). The therapeutic efficacy of paclitaxel was greatest for the ovarian carcinoma and the lymphoma, whose growth delays of 12.7 and 14.0 days, respectively, may be contrasted with the complete lack of measurable effect on tumor growth for the two squamous cell tumors. The correlation between tumor type and paclitaxel effect closely approximates that given in published reports on human tumors, where a range of effectiveness was observed. The highest objective response rates, apparent in breast and ovarian car-
cinomas, often being 30% or more (26-31), are most consistent with our results. Similar effectiveness has been demonstrated in the treatment of small-cell lung cancer (32), urothelial cancer (33), esophageal cancer (34), and head and neck cancer (35). In the intermediate range of effectiveness (20%) lie non-small-cell lung cancer (36) and high-grade astrocytoma (37). In the least effective category are melanoma (38,39), sarcoma (40), cervical carcinoma (41), and hormone-refractory prostate cancer (42). The failure of paclitaxel to induce growth delay in the murine squamous cell carcinomas appears to conflict with published clinical data. However, only two types of syngeneic murine squamous cell carcinoma have been examined; furthermore, considerable heterogeneity of response exists for human squamous cell carcinomas, an observation supported by findings in vitro (43).

The variability of response to paclitaxel raises questions and provides opportunities for addressing issues related to mechanisms of antitumor efficacy of the drug as well as its interaction with other cytotoxic agents. Discovery of a biological correlate for antitumor efficacy would be expected to answer important underlying mechanistic questions and would make it possible to predict the response in as yet untested tumor types as well as in individual tumors within a tumor type. Mitotic arrest has long been established as a hallmark cellular response to paclitaxel and one that underlies the rationale to use paclitaxel as a radiation sensitizer. In general, we observed a positive trend in which higher levels of induced mitotic arrest were associated with greater growth delay; however, this correlation was not significant, suggesting that a proportion of arrested cells survive. In vitro data from human leukemia cell lines have also suggested that mitotic arrest was unrelated to cytotoxicity (44). Further evidence is provided by the appearance of multinucleate cells after the peak of mitotic arrest in paclitaxel-treated squamous cell carcinomas and the previously published observation of polysomy in vitro (45).

The critical importance of apoptotic cell death in the heterogeneous response of murine tumors to radiation treatment was previously established (46). Since we observed apoptosis in murine tumors in response to paclitaxel (8,9), we sought to assess whether apoptotic cell death could be correlated with the heterogeneous response to paclitaxel in vivo. The finding that they were significantly correlated confirms the importance of apoptosis in the antitumor effect of this drug and lends support to the notion of failure to undergo apoptosis as a mechanism of drug resistance.

Why a tumor of one particular histologic type and not another would respond to paclitaxel by apoptotic cell death and why tumors within a histologic type would vary in their susceptibility to apoptosis are both unclear, and the answers to these questions are likely to be complicated. The ultimate outcome is the net result of the actions, and interactions, of various intrinsic genetic factors including c-myc, p53, and bcl-2 (47) and of various extrinsic factors including hormones, cytokines, and immune response effector cells (48). We have begun to study the functionality and temporal expression of a number of these factors in response to radiation and chemotherapeutic agents.

The correlation between antitumor effect and induced apoptosis suggests that induced apoptosis could be used to predict tumor response. However, although apoptosis induced by paclitaxel can be quantified, it may be both clinically undesirable and impractical to perform biopsies on tumors within 24 hours of commencing treatment. Instead, it seems more practical to determine the base-line level of apoptosis, since it was highly correlated with paclitaxel-induced apoptosis and with antitumor effect. The base-line apoptotic index is readily obtainable in the clinical context; indeed, preliminary work from our group has confirmed its usefulness in predicting the response of adenocarcinoma of the cervix to radiotherapy (49).

In addition, combining the observations on mitotic arrest and apoptosis may
allow prediction of the response of murine tumors to the combination of paclitaxel and radiation. We showed earlier that the radiopotentiating action of paclitaxel in the mouse mammary adenocarcinoma MCa-4 is attributable to paclitaxel-induced tumor reoxygenation (19). This tumor is sensitive to paclitaxel, with a growth delay of 4.8 days. Its histologic response is characterized by mitotic arrest and apoptotic cell death. In tumors, such as the SCC-VII squamous cell carcinoma, which do not manifest significant growth delay but demonstrate mitotic arrest only, early paclitaxel-induced tumor reoxygenation would not be expected, given the absence of any observable apoptotic response. Paclitaxel, however, may still act as a potentiator of the radiation response via its induction of mitotic arrest, as has been shown in vitro (13,15,17).

Our observations on the mitotic and apoptotic responses provide some insight into the underlying mechanism responsible for the variability seen among these syngeneic murine tumors. It is clear that apoptotic cell death is critical to the in vivo antitumor effect of paclitaxel, with those tumors that mount an apoptotic response showing significant growth delay. The finding of a strong correlation between base-line apoptosis and paclitaxel-induced apoptosis provides a useful marker for the capacity of a particular tumor to undergo apoptosis in response to a cytotoxic insult. Base-line apoptosis was also highly correlated with the subsequent antitumor effect of paclitaxel.

References

GETTING THE FACTS ON 5 A DAY
How Americans are doing when it comes to fruits and vegetables

Why eat five?

As the link between diet and overall health continues to gain attention, public awareness of the benefits of fruits and vegetables has expanded. In a recent survey, 1,003 people were asked how likely they thought it is that eating fruits and vegetables can help reduce the risk of several health conditions. Perceived health benefits most frequently mentioned were:

- Prevent Heart Disease
- Lose or Maintain Weight
- Prevent Cancer
- Lower Fat in Your Diet

59%  64%  48%  75%

Source: National Cancer Institute